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(54) Title: NOVEL ANTIOXIDANT FORMULATIONS AND METHODS FOR USING THEM

(57) Abstract

This invention relates to novel antioxidant formulations and methods for using them. The antioxidant formulations comprise a combination of a free radical scavenger (FRS), a radical scavenger recycler (RSR) and optionally, a radical formation inhibitor (RFI). The formulations of this invention may be used in pharmaceutical compositions, foodstuffs, food additives and dietary supplements. In addition, this invention relates to the use of the antioxidant formulations to inhibit oxidative damage and to treat and prevent disorders associated with oxidative damage caused by free radicals.

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NOVEL ANTIOXIDANT FORMULATIONS AND METHODS FOR USING THEM

TECHNICAL FIELD OF THE INVENTION

This invention relates to organic chemistry, inorganic chemistry, biochemistry and medicine. In particular, it relates to novel antioxidant formulations and methods for using them in the treatment and prevention of oxidative damage to cellular and other body components.

BACKGROUND OF THE INVENTION

The benefits of antioxidants to prevent the damaging effects caused by free radicals are well recognized.

Free radical oxidation has been postulated to play a role in many different pathogenic conditions, including atherosclerotic disease (Steinberg et al., N. Engl. J. Med. 320(14):915-24 (1989)). Free radical damage may occur in virtually any cellular constituent, including, without limitation, mitochondria, lysosomes, peroxisomes, the endoplasmic reticulum, nuclear and plasma membranes and numerous sites within the cytosol. This free radical damage can culminate in harmful mutations, cross-linkages, denaturation, and inactivation. For example, serum lipoproteins can become oxidized in vivo (Warso et al., J. Clin. Invest. 75:667-71 (1985)) and these may be more atherogenic than their unoxidized counterparts. Oxidized low density lipoprotein (LDL) can potentially promote atherogenesis by several mechanism including exerting a toxic effect on the arterial endothelium and chemotactically attracting and immobilizing monocytes and macrophages. Furthermore, the genetic machinery of the cell may be damaged, resulting in mutagenesis and carcinogenesis. In addition to atherosclerosis and cancer, free radicals have been implicated in a number of other disorders including kidney and liver conditions, inflammatory conditions (such as arthritis), circulatory problems, diabetes and signs of aging (such as collagen deterioration).

Numerous antioxidant formulations have been reported and many are commercially available. These formulations typically contain α-tocopherol (vitamin E) ascorbic acid (vitamin C), selenium, coenzyme Q10 and/or β-carotene (vitamin A) but can incorporate many other antioxidants as well. However, the amount of each individual agent is often not optimal, it either being present in unnecessary excess or below the effective dose. In addition, each formulation may not contain the most synergistic combination of antioxidant agents. This may require the user to take more than one antioxidant formulation to obtain maximum benefit. Furthermore, these formulations are often characterized by poor lipid solubility, preventing their effectiveness in lipid environments, such as the lipid membrane.

Accordingly, there remains a need for new antioxidant formulations including those that provide an effective dose of synergistic lipid- and water-soluble antioxidants.

SUMMARY OF THE INVENTION

The novel antioxidant formulations of this invention fill the unmet need described above.

It is an object of this invention to provide an antioxidant formulation comprising an effective amount of a free radical scavenger (FRS), a radical scavenger recycler (RSR) and optionally, a radical formation inhibitor (RFI).

It is another object of this invention to provide methods for using the antioxidant formulations of this invention to inhibit oxidative damage and to treat and prevent disorders associated with oxidative damage caused by free radicals.

Thus, in one aspect, this invention is directed to an antioxidant formulation comprising tocotrienol or tocotrienol mixtures and one or more radical scavenger recyclers capable of recycling the tocotrienol or tocotrienol mixture.

In another aspect of this invention, the radical scavenger recycler is selected from the group consisting of α-lipoic acid, ascorbic acid, salts of either acid, derivatives of either acid and mixtures thereof.

In yet another aspect of the present invention, the radical scavenger recycler comprises a mixture of α-lipoic acid and ascorbic acid or a water-soluble derivative thereof

A formulation comprising a mixture of α-lipoic acid, ascorbic acid or a water soluble derivative thereof and a lipid-soluble ascorbic acid derivative is an aspect of this invention.

It is an aspect of this invention that the optional lipid-soluble ascorbic acid derivative is ascorbyl palmitate.

It is likewise an aspect of this invention that the above antioxidant formulation further comprise a radical formation inhibitor.

A further aspect of this invention is that the radical formation inhibitor is pyruvate.

Still another aspect of this invention is an antioxidant formulation comprising:

one or more free radical scavengers, one or more radical scavenger recyclers and one or more radical formation inhibitors.

The free radical scavenger in the formulation immediately above comprises tocotrienol or tocotrienol mixture in another aspect of this invention.

Furthermore, in the above formulation, the radical scavenger recycler is selected from the group consisting of α-lipoic acid, ascorbic acid or a water soluble derivative thereof and mixtures thereof in another aspect of this invention.

In another aspect of this invention, the preceding formulation contains as the radical scavenger recycler a mixture of α-lipoic acid, a water-soluble ascorbic acid derivative and a lipid-soluble ascorbic acid derivative.

It is an aspect of this invention that the lipid-soluble ascorbic acid derivative is ascorbyl palmitate.

A presently preferred embodiment of this invention is that the radical formation inhibitor in the preceding formulation is pyruvate.

In the above formulations, the amount of tocotrienol or tocotrienol mixture is from about 1 to about 1000 mg in a further aspect of this invention.

It is yet another aspect of this invention that the formulation described herein comprises between about 1 and about 1000 mg of α-lipoic acid and

between about 10 and about 1000 mg of ascorbic acid or a water-soluble derivative thereof.

It is an aspect of this invention that the above formulation also contain from about 1 to about 500 mg of a lipid soluble ascorbic acid derivative.

A formulation comprising between about 20 and about 60 mg of tocotrienol or tocotrienol mixture, between about 400 and about 600 mg of ascorbic acid and between about 100 and about 300 mg of α-lipoic acid, is a further aspect of this invention.

Between about 40 and about 80 mg of lipid soluble ascorbic acid is also added to the formulation immediately above in a further aspect of this invention.

A presently preferred embodiment of this invention is a formulation comprising between about 10 and about 30 mg of tocotrienol or tocotrienol derivative, between about 200 and about 300 mg of ascorbic acid, between about 50 and about 150 mg of α-lipoic acid and between about 400 and about 600 mg of pyruvate.

Still another aspect of this invention is the above presently preferred embodiment further comprising between about 20 and about 40 mg of a lipid soluble ascorbic acid derivative.

Another aspect of this invention is a formulation comprising from about 30 to about 50 mg of tocotrienol or tocotrienol mixture, about 600 to about 700 mg of pyruvate, about 75 to about 125 mg of α-lipoic acid and from about 90 to about 110 mg of ascorbic acid or a water soluble derivative thereof.

It is an aspect of this invention that the preceding formulation also comprise from about 60 to about 70 mg of ascorbyl palmitate.

A formulation comprising from about 10 to about 30 mg of tocotrienol or tocotrienol mixture, 300 – 400 mg of pyruvate, 25 – 75 mg α-lipoic acid, from about 40 to about 60 mg ascorbic acid, from about 0.1 to about 10.0 mg of N-acetylserotonin and from about 1000 to about 6000 mg of L-arginine is yet another aspect of this invention.

It is a presently preferred embodiment of this invention that the L-arginine in the above formulation is in the form of a sustained release composition.

In another aspect of this invention, N-acetlyserotonin in the above formulation is replaced with from about 0.1 to about 10 mg of melatonin.

It is also an aspect of this invention that the preceding formulation additionally comprises from about 25 to about 35 mg acorbyl palmitate

A method for using the antioxidant formulations of this invention to inhibit oxidative damage in a patient comprising administering to a patient in need thereof a composition comprising said formulation is also an aspect of this invention.

It is an aspect of this invention that the above formulations further contain between about 25 and about 35 mg of ascorbyl palmitate.

A method for inhibiting oxidative damage in a patient by administering a formulation of this invention to the patient is a further aspect of this invention.

A method for treating or preventing a condition, disease or disorder associated with free radical oxidative damage comprising administering a formulation of this invention to a patient in need thereof is yet another aspect of this invention.

In a presently preferred embodiment of this invention, a combination of two formulations is administered to a patient in need thereof wherein the first formulation, administered in the morning, comprises about 30 to about 50 mg of tocotrienol or tocotrienol mixture, about 600 to about 700 mg of pyruvate, about 75 to about 125 mg of α-lipoic acid and from about 90 to about 110 mg of ascorbic acid and the second formulation, administered in the evening comprises from about 10 to about 30 mg of tocotrienol or tocotrienol mixture, from about 300 to about 400 mg of pyruvate, from about 25 to about 75 mg α-lipoic acid, from about 40 to about 60 mg ascorbic acid, from about 0.1 to about 10.0 mg of N-acetylserotonin and from about 1000 to about 6000 mg of L-arginine.

It is likewise an aspect of this invention that the oxidative damage associated condition, disease or disorder which may be treated or prevented by use of the formulations of this invention is selected from the group consisting of atherosclerotic diseases, kidney and liver conditions, inflammatory conditions, circulatory problems, diabetic conditions, signs of aging, neurological disorders, eye diseases and exposure to environmental pollution.

In yet another aspect of this invention, the formulations herein may be used to inhibit cytokines involved in the inflammatory response.

It is a further aspect of this invention that the cytokine inhibited is selected from the group consisting of IL-1, IL-6, IL-8 and TNS-α.

Finally, it is an aspect of this invention that the formulations herein may be used to inhibit or reduce C-reactive protein in a patient.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the following definitions apply (unless expressly noted to the contrary):

"Antioxidant agent" refers to a free radical scavenger (FRS), a radical scavenger recycler (RSR) or a radical formation inhibitor (RFI) according to this invention.

"Effective amount" refers to an amount sufficient to inhibit oxidative damage or to treat or prevent disorders associated with oxidative damage caused by free radicals. An effective amount of an antioxidant agent included in a particular formulation of this invention should be determined in combination with the other antioxidant agents included in that formulation. When no specific amounts are indicated, an effective amount is to be presumed.

"Formulation" as used herein refers to a preparation for administration via any acceptable route known to those of ordinary skill in the art. Such routes include, but are not limited to oral, parenteral, transdermal, intravenous or topical administration. "Formulation" encompasses pharmaceutical compositions as well as dietary supplements, foodstuffs, food additives and the like.

"Free radical scavenger" or "FRS" refers to an agent characterized by an ability to neutralize free radicals and/or prevent free radical chain propagation. For example, a FRS according to this invention may prevent the peroxidation of lipid free radicals by protonation of a lipid free radical or lipid radical alkoxide. FRSs according to this invention include, but are not limited to, tocopherols, tocotrienols, glutathione, glutathione derivatives and other amino acids and amino acid derivatives, flavanoids (such as catechins, quercetin, rutin, anthocyanidins, proanthocyanidins (such as pycnogenols and spiraeoside) and flavanoid blends, isoflavanoids (such as isoquercitin) and isoflavanoid blends, phenolic acids,

selenium, coenzyme Q10, vitamin B complex, Vitamin B-1 (thiamine),vitamin B-2(riboflavin), vitamin B-3, niacin (including nicotinic acid and nicotinamide) and carotenoids (such as beta-carotene and lycopene).

"Radical formation inhibitor" or "RFI" refers to an agent characterized by an ability to inhibit the endogenous formation of damaging free radicals. RFIs according to this invention include, but are not limited to pyruvic acid and derivatives thereof (including pyruvate salts and esters) and arginine and derivatives thereof (including salts and amide esters). Preferably, the RFI for use in the formulations of this invention is selected from the group consisting of sodium pyruvate, calcium pyruvate, a pyruvyl amino acid or amino acid amide. In some cases, the radical formulation inhibitor may also possess the activity of a free radical scavenger. However, for the purpose of this disclosure, agents possessing the activity of a RFI shall be considered RFI agents and not FRS agents.

"Radical scavenger recycler" or "RSR" refers to an agent characterized by an ability to recycle one or more of the FRSs according to this invention. For example, in the case where tocotrienol or tocopherol is included as a FRS, the corresponding RSR may be α-lipoic acid and/or ascorbic acid derivatives (including salts and esters thereof) and/or glutathione and derivatives thereof. Similarly, α-lipoic acid has the ability to recycle spent ascorbic acid and ascorbic acid derivatives (including salts and esters thereof) and coenzyme Q10. Therefore, α-lipoic acid may be included as a RSR in formulations including ascorbic acid, ascorbic acid derivatives and/or coenzyme Q10. Preferred ascorbic acid derivatives for use in the formulations of this invention include ascorbyl palmitate and other lipid soluble forms of ascorbic acid. In some cases, the radical scavenger recycler may also possess the activity of a free radical scavenger. However, for the purpose of this disclosure, agents possessing the activity of a RSR shall be considered RSR agents and not FRS agents, provided that the corresponding FRS is included in the same formulation.

"Tocotrienol" refers to a compound possessing the following three structural characteristics: (1) a hydrogen donor group (or a group that can be hydrolyzed to a hydrogen donor group) attached to an aromatic ring system; (2) a

side chain attached to the aromatic ring system comprising one or more isoprenoid or isoprenoid-like units and (3) a methylene unit or a functional group having at least one lone pair of electrons positioned adjacent to the atom to which the side chain is attached to the aromatic ring, said electrons being conjugated to the aromatic ring system (preferably CH_2, C=O, CHOH, O, S or NH). Preferred tocotrienols for use in this invention are those which are naturally occurring (including α-, β-, γ-, δ- tocotrienol). The term "tocotrienol" refers to a single tocotrienol or a mixture of tocotrienols. These naturally occurring tocotrienols may be conveniently isolated from biological materials or synthesized from commercially available starting material. The tocotrienols for use in the methods of this invention may be obtained from biological materials that have been stabilized and extracted, such as by the processes described in PCT publication WO 91/17985 and co-pending US application 08/583,232 (the entire disclosures of which are hereby incorporated by reference). Examples of preferred biological materials from which naturally-occurring tocotrienols can be obtained and examples of synthetic tocotrienols and how to make them are described in US patents 5,591,772 and 5,821,264 and PCT publication WO 91/17985 (the entire disclosures of which are hereby incorporated by reference). Preferred biological materials from which the tocotrienols useful in this invention may be obtained include stabilized brans, particularly stabilized rice bran.

The term "tocotrienol mixture" as used herein, refers to two or more of the following: one or more naturally-occurring tocotrienols, one or more synthetic tocotrienols, one or more naturally-occurring tocopherols and one or more naturally-occurring free fatty acids.

The tocotrienol mixture of this invention may optionally be treated to remove free fatty acids. An example of how this may be accomplished is provided in the Examples section, below.

The term "about" as used herein with regard to the amounts of materials in a formulation means within ±10%. For example, if a value of, say, 100 is given to a parameter, that value may vary from 90 to 110 and still be with the scope of this invention.

By "essentially free of free fatty acids" is meant that a treated tocotrienol mixture contains less than 10% free fatty acids, preferably less than 5% and most preferably less than 2%.

This invention expressly encompasses the "prodrug" forms of tocotrienols and other antioxidants included in the formulations of this invention. Upon administration, such a prodrug undergoes biotransformation to their active form. Prodrugs include the salt and esterified forms of the tocotrienols and other antioxidants that may be included in the formulations of this invention.

The tocotrienols and other antioxidants included in the formulations of this invention that have at least one chiral center may be in isomerically pure form or may be a mixture of isomers. For example, the tocotrienols of this invention may exist as the d- or l-isomer or the d,l-racemic mixture. The naturally occurring isomer (usually the d-isomer) and the d,l-racemic mixture are preferred.

All literature cited herein is incorporated by reference in its entirety as if fully set forth herein.

Without wishing to be bound to any particular theory, it is presently thought that the antioxidant formulations according to this invention benefit particularly from the combination of free radical scavanger properties of one or more FRSs and the recycling properties of one or more RSRs. Some of the RSRs useful in this invention (such as α-lipoic acid and ascorbate) may also have independent free radical scavenging abilities. Advantageously, use of an RSR, together with the FRS that it recycles, permits use of less of the FRS while also resulting in improved antioxidant activity of the formulation. This reasoning is especially applicable to one of the preferred RSR/FRS combinations according to this invention: tocotrienol as the FRS and α-lipoic acid (or a derivative thereof) and/or ascorbic acid (or a derivative thereof) as the RSR.

Inclusion of a RFI provides an additional benefit to the formulations of this invention by inhibiting the initial formation of damaging free radicals. The RFIs that may be used in the formulations of this invention (such as pyruvate and pyruvate salts and esters) may also have independent free radical scavenging properties. Advantageously, the use of one or more RSRs, FRSs and RFIs in combination may form a synergistic mixture with improved antioxidant activity.

Such appears to be the case in one of the presently preferred RSR/FRS/RFI combinations according to this invention, that is, tocotrienol as the FRS, α-lipoic acid (or a derivative thereof) and/or ascorbic acid (or a derivative thereof) as the RSR and pyruvate (or a derivative thereof) as the RFI.

A presently preferred RSR/FRS combination according to this invention comprises an effective amount of tocotrienol or a tocotrienol mixture as the FRS and α-lipoic acid (or a derivative thereof) and/or ascorbic acid (or a derivative thereof) as the RSR. More preferably, the RSR/FRS combination comprises an effective amount of tocotrienol or tocotrienol mixture as the FRS, α-lipoic acid (and/or a salt thereof) as one RSR and ascorbic acid (and/or a salt or water-soluble ester thereof) as a second RSR. In another aspect of this invention, this presently preferred embodiment may also optionally contain a lipid-soluble ascorbyl ester (such as an ascorbyl fatty acid ester, e.g. ascorbyl palmitate) as a third RSR. This latter combination results in a formulation which is both water- and lipid-soluble and which, therefore, would be expected to exhibit antioxidant activity in a number of physiological environments. The inclusion of tocotrienol is especially advantageous given its impressive range of biological activity. Tocotrienols are more potent antioxidants than the commonly used vitamin E forms (tocopherols) and can be recycled from their inactivated molecular forms back to their active forms by certain RSRs, including ascorbic acid and α-lipoic acid, which also have antioxidant actions of their own. There are currently no antioxidant products on the market that combine tocotrienols and their recycling agents, ascorbic acid and α-lipoic acid, in a single formulation. Furthermore, the inclusion of a water-soluble RSRs (e.g., α-lipoic acid, ascorbic acid and/or salts thereof) and lipid-soluble RSRs (ascorbyl palmitate and α-lipoic acid(which is soluble in both aqueous and lipid environments)) can further enhance the formulation by distributing the tocotrienol RSRs within the body in both the aqueous and lipid domains since recycling of tocotrienols can occur in both domains.

A presently preferred RSR/FRS/RFI combination according to this invention comprises an effective amount of tocotrienol as the FRS, α-lipoic acid (or a derivative thereof) and/or ascorbic acid (or a derivative thereof) as the RSR

and pyruvate (or a salt or ester thereof) as the RFI. More preferably, the RSR/FRS/RFI combination comprises tocotrienol as the FRS, α-lipoic acid (and/or a salt thereof) as one RSR, ascorbic acid (and/or a salt thereof) as a second RSR and a pyruvic acid salt (such as calcium pyruvate) as the RFI. Optionally, this formulation may also contain ascorbyl palmitate as a lipid soluble third RSR

Formulations of this invention are prepared by combining an effective amount of one or more RSR, FRS and optionally, RFI agents with an acceptable carrier. Acceptable carriers are those which are non-toxic for the mode and level of administration and which do not destroy the activity of the active components of the formulation. Acceptable carriers for the formulations of this invention are well known to those of ordinary skill in the art.

The formulations of this invention may be used to inhibit oxidative damage and to treat and prevent disorders associated with oxidative damage caused by free radicals. Such disorders include, but are not limited to atherosclerotic disease, cancer, kidney and liver conditions, diabetes, inflammatory conditions (such as arthritis), circulatory problems, signs of aging (such as collagen deterioration), neurological disorders (such as Alzheimer's disease and Parkinson's disease), eye diseases (such as cataracts) and exposure to environmental pollution (such as ozone exposure).

The use of the formulations of this invention with respect to the treatment of oxidative stress and AGE formation in diabetic patients, while not intended to be limiting in any way on the scope of this invention, is illustrative.

Diabetes mellitus is a chronic disease characterized by either a lack of insulin (insulin dependent diabetes) or by a reduced responsiveness of tissues to insulin (non-insulin dependent diabetes). Insulin is required for the transport of glucose from the blood into cells when it is metabolized as a source of energy. A decreased insulin. effect, whether it result from a lack of insulin or from a deficient insulin response, reduces the clearance of glucose from the blood and produces a chronic elevation of blood glucose (hyperglycemia). This reactive high blood glucose has been shown to cause oxidative stress. Furthermore, prolonged hyperglycemia causes detrimental changes in, among others things, various proteins, lipids and nucleic acid constituents as the result of glycation.

Excessively available sugar carbonyl groups from high glucose can result in the glycation of free amino acid groups of proteins. The ultimate effect of this glycation is the generation of high molecular weight protein aggregates and other entities, known generally as advanced glycation end products (AGEs). The production of AGEs has been suggested to also require, in addition to high glucose, the presence of free radical species such as the superoxide anion ($O_2^{\cdot-}$).

Superoxide anion is a member of a group of materials known a reactive oxygen species (ROS). In diabetic patients, an enzyme known as superoxide dismutase, which can inactivate superoxide anion, is present in amount substantially lower than in non-diabetics. Thus, superoxide anion and other ROS such as hydrogen peroxide and hydroxyl radical are more readily able to cause damage to sensitive cellular components. For instance, ROS can react with nitric acid to form the extremely potent oxidants, peroxynitrite and peroxynitous acid. In addition ROS are connective link between high glucose and elevated inflammatory cytokine production and pathogenic cellular damage and other diabetes-related complications. The need for effective antioxidants which can control the initial formation of oxygen free radicals and derivatives thereof is clear; the present invention provide formulations which have the ability to contain such deleterious free radicals.

That is, the formulations of this invention comprise tocotrienol or tocotrienol mixture, which act as oxygen radical scavengers as well as radical inhibitors that can prevent the initial formation of oxygen radicals. They also contain ascorbate, either in a water-soluble form for the scavenging of free radicals in aqueous environments or in a water-soluble and a lipid soluble form for the scavenging of free radicals in both aqueous and lipid environments. The formulation herein also comprise alpha lipoic acid, which, together with ascorbate, recycles the tocotrienol or tocotrienol mixture. Finally, the formulations herein contain pyruvate which is yet another free radical scavenger. Thus, the formulation of the present invention provide an effective, broad spectrum therapy for the prevention and treatment of oxidative stress and AGEs as well as other free-radical related damage to cellular components.

The formulations of this invention may be administered by any acceptable means to inhibit oxidative damage and to treat and prevent disorders associated

with oxidative damage caused by free radicals. For example, formulations of this invention may be administered orally, topically, transdermally, parenterally, intravenously or by inhalation. These formulations may be produced so as to impart a time-released benefit. Oral compositions may take the form of tablets, capsules, caplets, emulsions, liposomes, suspensions, powders and the like. Topical compositions include, but are not limited to, gels, lotions and creams. Parenteral compositions take the form of sterile solutions and emulsions and the like. Intravenous compositions include, but are not limited to sterile solutions. The preferred route of administration of the formulations according to this invention is oral.

Effective dosage levels for each of the antioxidants may be determined by methods known to those of skill in the art. In some cases, guidance may be gleaned from published recommended daily allowances. Preferred dosage forms incorporate formulations designed for once a day, oral administration. Typically, dosage levels will range between about 1 and about 1000 mg of tocotrienol or tocotrienol mixture per dose (more preferably, between about 10 and 500 mg of tocotrienol or tocotrienol mixture per dose and most preferably, between 10 and 100 mg/dose). For ascorbic acid, dosage levels will typically range between about 10 and about 1000 mg per dose (more preferably, between about 50 and 500 mg per dose and most preferably, between 100 and 500 mg/dose). For lipid soluble ascorbic acid derivatives, dosage levels will typically range between about 1 and about 500 mg per dose (more preferably, between about 10 and 100 mg per dose and most preferably, between 10 and 50 mg/dose). For α-lipoic acid, dosage levels will typically range between about 1 and about 1000 mg per dose (more preferably, between about 10 and 600 mg per dose and most preferably, between 50 and 500 mg/dose). For pyruvate, dosage levels will typically range between about 100 and about 10000 mg per dose (more preferably, between about 100 and 2000 mg per dose and most preferably, between 100 and 1000 mg/dose). Multiple doses may be required over a period of time to obtain maximum benefit. Specific dosage and treatment regimens will depend upon factors such as the patient's overall health status, the severity and course of the patient's disorder or disposition thereto and the judgment of the treating physician. Higher or lower doses may be employed as needed.

It may be desirable to administer different formulation of the compounds of this invention at different times. For example, melatonin (or its precursor, N-acetylserotonin) is a strong contributing antioxidant and helps to up-regulate the enzyme superoxide dismutase, a key cellular component that protects against damage to cellular components by superoxide. L-arginine, on the other hand, provides a substrate for cooperatively reducing the levels of synthesized superoxide. Both melatonin (or N-acetylserotonin) and L-arginine have low cycling levels at night due to the natural circadian rhythm. Thus, a formulation comprised of tocotrienols or tocotrienol mixtures, pyruvate, α-lipoic acid and ascorbic acid could be administered in the morning while, in the evening, when the natural circadian rhythm results in low cycling levels of melatonin and L-arginine, a formulation comprised of tocotrienols or a tocotrienol mixture, pyruvate, α-lipoic acid, ascorbic acid, N-acetylserotonin and L-arginine could be administered. The latter formulation should be particularly beneficial to diabetics since the levels of both the compounds discussed above are often found to be undesirably low in such individuals.

Cytokines comprise a large group of proteins may of which are involve in the inflammatory response of an organism to injury or disease. Interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF-α) are the cytokines which are most involved with the inflammatory response. The formulations of this invention are effective to inhibit or reduce the activity of these cytokines and thereby reduce inflammation in a patient. Such reduction in inflammation may be particularly important in that cytokine-mediated inflammation has been related to atherosclerosis.

C-reactive protein is another protein which has been related to the inflammatory response. The protein is so called because of its ability to bind and to be precipitated by the C-polysaccharide of Steptococcus pneumoniae. Due to its relationship to inflammation and the ability of the formulations of this invention to inhibit or reduce inflammation, it is expected that the formulations herein will inhibit or reduce the production of C-reactive protein as well as cytokines.

EXAMPLES

In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

The following formulations of this invention may be produced in the form of capsules, tablets, drinks, drink mixes, snack bars, foodstuffs, dietary supplements or in any other orally acceptable administration form which, based on the disclosures herein would be apparent to those skilled in the art. Any such oral administrative approach is within the scope of this invention.

Formulation I:

Tocotrienol or tocotrienol mixture	20 – 60 mg
Ascorbic acid	400 – 600 mg
α-Lipoic acid	100 – 300 mg

To the above formulation, 40 – 80 mg of ascorbyl palmitate (or other lipid soluble ascorbic acid derivative), may be added.

Formulation II:

Tocotrienol or tocotrienol mixture	10 – 30 mg
Ascorbic acid	200 – 300 mg
α-Lipoic acid	50 – 150 mg
Pyruvate	400 – 600 mg

To the above formulation, 40 – 80 mg of ascorbyl palmitate (or other lipid soluble ascorbic acid derivative) may be added.

Formulation III:

Tocotrienol or tocotrienol mixture	40 – 50 mg
Ascorbic acid	90 – 110 mg
α-Lipoic acid	90 – 110 mg
Pyruvate	475 – 525 mg

Formulation IV:

Tocotrienol or tocotrienol mixture	5 – 15 mg
Ascorbic acid	40 – 60 mg
α-Lipoic acid	45 – 55 mg
Pyruvate	325 – 375 mg
N-Acetylserotonin	0.5 – 1.5 mg
L-Arginine	1250 – 1750 mg

In Formulation IV, the N-acetylserotonin may be replaced with 0.1 – 5 mg of melatonin.

The antioxidant efficacy of these and other formulations of this invention can be evaluated by methods known to those of ordinary skill in the art, including: plasma thiobarbituric acid-reacting substances (TBARs), oxygen radical absorbance capacity (ORAC), randox trolox-equivalent antioxidant capacity (Randox-TEAC), ferric reducing ability (FRAP) assay, and by measuring malondialdehyde or isoprostane levels.

Preparation of free fatty acid free tocotrienol mixtures:

The following preparation of free fatty acid free tocotrienol mixtures is provided as an example only and is not intended and should not be construed as limiting the scope of this invention in any manner. Based on the disclosures herein, other methods for preparing free fatty acid free tocotrienols mixtures will become apparent to those skilled in the art; such methods are within the scope of this invention.

Deodorized rice bran oil (commercially available) is esterified with methanol/H^+ to convert the free fatty acids to methyl esters. These methyl esters are relatively volatile and can be removed by distillation. Any heavy residues are then also removed. A mixture of tocopherols, tocotrienols and sterol esters remains. The sterol esters are removed by crystallization from a hydrocarbon solvent, typically hexane. The solvent is then removed, leaving the tocopherol/tocorientol fraction as an approximately 30% solution in neutral oils, primarily glycerides.

Free radical scavenging potency

The following formulation was tested for free radical scavenging potency:

Formula V:

Tocotrienol mixture	5.0 g
Ascorbic acid	3.3 g
Palmityl ascorbate	1.0 g
α-Lipoic acid	3.3 g
Sodium Pyruvate	16.7 g

The potency of the above formulation was compared to that of pure dl-α-tocopherol.

The automated assay of oxygen radical absorbance capacity using a COBAS FARA II centrifugal analyzer as described by Cao, et al., *Clin. Chem.*, **1995**, 41(12):1738-44, was used.

The test material was dissolved in 0.05% sodium dodecyl sulfate and then placed in the assay cuvettes. The fluorescence indicator protein, β-phycoerythrin was then added to the cuvettes followed by the peroxyl radical generator, 2,2'-azobis(2-amidinopropane). In the absence of an efficient radical scavenger, peroxyl radicals generated by the 2,2'-azobis(2-amidinopropane) will interact with the β-phycoerythrin and thereby decrease its normal fluorescence emission. Thus, a decrease in the loss of fluorescence is a direct measure of the ability of a radical scavenger to remove peroxyl radicals from the solution. The activity of the formulation is expressed in terms of the equivalent activity of the vitamin E derivative Trolox.

Material	Activity
	μmoles TE/gram*
dl-α-tocopherol	357
Formulation V	835
Tocotrienol mixture**	282

* Activity expressed as micromoles of Trolox (TE) equivalent per gram.

** This tocotrienol mixture consisted of a mixture of α, γ and δ tocopherols and tocotrienols (30%) and naturally-occurring rice bran oil free fatty acids (70%).

As can be readily seen, Formulation V is a substantially superior radical scavenger than either dl-α-tocopherol or a tocotrienol mixture alone.

While we have described a number of embodiments of this invention, it is apparent that our basic constructions may be altered to provide other embodiments which utilize the formulations and methods of this invention. Such alterations are within the scope of this invention. Other embodiments of this invention are provided in the claims which follow.

WHAT IS CLAIMED:

1. An antioxidant formulation comprising tocotrienol or tocotrienol mixture and one or more radical scavenger recyclers capable of recycling said tocotrienol or tocotrienol mixture.

2. The formulation according to claim 1, wherein the radical scavenger recycler is selected from the group consisting of α-lipoic acid, α-lipoic acid salts, derivatives of α-lipoic acid, ascorbic acid, ascorbic acid salts, derivatives of ascorbic acid and mixtures of these compounds.

3. The formulation according to claim 1, wherein the radical scavenger recycler comprises α-lipoic acid and ascorbic acid or a water-soluble derivative thereof.

4. The formulation according to claim 1, wherein the radical scavenger recycler comprises α-lipoic acid, ascorbic acid or a water soluble derivative thereof and a lipid-soluble ascorbic acid derivative.

5. The formulation according to claim 4, wherein the lipid-soluble ascorbic acid derivative is ascorbyl palmitate.

6. The formulation according to any one of claims 1-5, further comprising a radical formation inhibitor.

7. The formulation according to claim 6, wherein the radical formation inhibitor is pyruvate.

8. An antioxidant formulation comprising:

one or more free radical scavengers;

one or more radical scavenger recyclers; and

one or more radical formation inhibitors.

9. The formulation according to claim 8, wherein the free radical scavenger is tocotrienol or tocotrienol mixture.

10. The formulation according to claim 8, wherein the radical scavenger recycler is selected from the group consisting of α-lipoic acid, ascorbic acid or a water soluble ascorbic acid derivative and mixtures thereof.

11. The formulation according to claim 9, wherein the radical scavenger recycler is α-lipoic acid, ascorbic acid or a water-soluble derivative thereof and a lipid soluble ascorbic acid derivative.

12. The formulation according to claim 10, wherein the lipid-soluble ascorbic acid derivative is ascorbyl palmitate.

13. The formulation according to claim 9, wherein the radical formation inhibitor is pyruvate.

14. The formulation according to claim 1 or 8, comprising between about 1 and about 1000 mg of tocotrienol or tocotrienol mixture.

15. The formulation according to claim 3 or 10, comprising between about 1 and about 1000 mg of α-lipoic acid and between about 10 and about 1000 mg of ascorbic acid or a water-soluble derivative thereof.

16. The formulation according to claim 15, further comprising between about 1 and about 500 mg of a lipid-soluble ascorbic acid derivative.

17. An antioxidant formulation, comprising:

between about 20 and about 60 mg of tocotrienol or tocotrienol mixture;

between about 400 and about 600 mg of ascorbic acid; and,

between about 100 and about 300 mg of alpha lipoic acid.

18. The formulation according to claim 17, further comprising between about 40 and about 80 mg of a lipid soluble ascorbic acid derivative.

19. An antioxidant formulation, comprising:

between about 10 and about 30 mg of tocotrienol or tocotrienol mixture;

between about 200 and about 300 mg of ascorbic acid or a water soluble derivative thereof;

between about 50 and about 150 mg of α-lipoic acid; and

between about 400 and about 600 mg of pyruvate.

20. The formulation according to claim 19, further comprising between about 20 and about 40 mg of a lipid soluble ascorbic acid derivative.

21. A formulation comprising:

between about 30 and about 50 mg of tocotrienol or tocotrienol mixture;

between about 600 and about 700 mg of pyruvate;

between about 75 and 125 mg of α-lipoic acid; and,

between about 90 and about 110 mg of ascorbic acid or a water-soluble derivative thereof.

22. The formulation according to claim 21, further comprising between about 60 and about 70 mg of ascorbyl palmitate.

23. An antioxidant formulation comprising:

between about 10 and about 30 mg of tocotrienol or tocotrienol mixture;

between about 300 and about 400 mg of pyruvate;

between about 25 and about 75 mg of α-lipoic acid;

between about 40 and about 60 mg of ascorbic acid or a water soluble ascorbic acid derivative;

between about 0.1 and about 10 mg of N-acetylserotonin; and,

between about 1000 and about 6000 mg of L-arginine.

24. The formulation according to claim 23 wherein said L-arginine comprises a sustained-release composition.

25. A formulation comprising:

between about 10 and about 30 mg of tocotrienol or tocotrienol mixture;

between about 300 and about 400 mg of pyruvate;

between about 25 and about 75 mg of α-lipoic acid;

between about 40 and about 60 mg of ascorbic acid or a water soluble ascorbic acid derivative;

between about 0.1 and about 10 mg of melatonin; and,

between about 1000 and about 6000 mg of L-arginine.

26. The formulation according to either one of claims 23 or 25, further comprising between about 25 and about 35 mg of ascorbyl palmitate.

27. A method for inhibiting oxidative damage, comprising administering to a patient in need thereof said antioxidant formulation according to any one of claims 1, 8, 17, 19, 21, 23 or 25.

28. A method for treating or preventing a condition, disease or disorder associated with oxidative damage, comprising administering to a patient in need thereof said antioxidant formulation according to any one of claims 1, 8, 17, 19, 21, 23 or 25.

29. A method for treating or preventing a condition, disease or disorder associated with oxidative damage, comprising:

administering said antioxidant formulation of claim 21 in the morning to a patient in need thereof; and,

administering said antioxidant formulation of either claim 23 or claim 25 to said patient in the evening.

30. The method according to either claim 28 or claim 29, wherein said condition, disease or disorder is selected from the group consisting of atherosclerotic diseases, kidney and liver conditions, inflammatory conditions, circulatory problems, diabetic conditions, signs of aging, neurological disorders, eye diseases and exposure to environmental pollution.

31. A method for the inhibition or reduction of cytokines comprising administering a formulation of any one of claims 1, 8, 17, 19, 21, 23 or 25 to a patient in need thereof.

32. The method of claim 31, wherein said cytokine is selected from the group consisting of IL-1, IL-6, IL-8 and TNF-α.

33. A method for the inhibition or reduction of C-reactive protein comprising administering a formulation of any one of claims 1, 8, 17, 19, 21, 23 or 25 to a patient in need thereof.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/07733

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 31/355
US CL : 514/458
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/458, 424/401, 549/401

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EAST, STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,376,361 A (PERRICONE) 27 December 1994, see entire document	1-33
Y	US 5,591,772 A (LANE et al.) 07 January 1997, see entire document.	1-33
Y	US 5,709,868 A (PERRICONE) 20 January 1998, see entire document	1-33
Y	US 5,545,398 A (PERRICONE) 13 August 1996, see entire document.	1-33
Y,P	US 5,952,373 A (LANZENDORFER et al.) 14 September 1999, see entire document	1-33
Y	US 5,834,513 A (PTCHELINTSEV et al.) 10 November 1998, see entire document	1-33

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance.	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
08 MAY 2000	24 MAY 2000
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